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Original Article

ANALYSIS OF GROWTH AND SPORULATION OF FILAMENTOUS FUNGI IN DIFFERENT LIQUID MEDIA

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ABSTRACT

Composition of culture medium plays a significant role in growth and sporulation of experimental microorganisms. Preparation of most suitable culture media is one of the prerequisites to investigate them. The aim of the present investigation was, to explore four different culture broth namely modified Sabouraud's medium, Richard's medium, Yeast extract medium and Czapek's medium for maximum growth and sporulation of selected keratinophilic (Chrysosporium indicum and Trichophyton terrestre) and dermatophytic fungi (T. rubrum and T. simii), collected from Jaipur district. Initial pH of all media was maintained at 7.5. During present study, the best sporulation was achieved in SDM, followed by RM in all the test fungi. T. simii and T. rubrum showed maximum growth in SDM broth, while in case of T. terrestre and C. indicum, better growth were achieved in Richard's medium. CzM was found to be less suitable for the growth and sporulation of almost all the selected fungi. Final pH of all mediums was found to be changed from initial pH. The information generated will facilitate Mycological research on the fungus causing superficial infection of human being.

KEYWORDS: Media, Sporulation, Growth, pH & Dermatophytes

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INTRODUCTION

Infection caused by fungi in man and animal is common throughout the world. Fungi caused both superficial and internal Mycoses. The majority of superficial infections are caused by a closely related group of keratinophilic fungi called dermatophytes. The keratinophilic and dermatophytic fungi are unique in the sense that they require and utilize keratin for the growth. Therefore, infection by this group of fungi is confined to superficial integument including the outer stratum corneum of the skin, the nails and hair (Jain et al., 2008; Jain et al., 2014). Keratinophilic fungi are generally considered as soil saprophytes (Ajello 1953; 1956). They occur on cornfield debris in the soil and degrade keratin and keratinous material. Therefore, they play an important ecological role in decomposing such residue. Various keratinophilic fungal species have a very important role in the degradation of keratins wastes and subsequent production of useful products like animal feeds and fertilizers (Sharma et al., 2011). Fungi, being saprophytic grow up in different habitats in the environment and require numerous nutritional components for growth and reproduction (Albert et al., 2012),

A variety of biochemical and physiological factors has significant impact on the growth and development in microorganisms, and manage their metabolism towards increase or decrease growth with certain morphological measurable changes. Accordingly, the fungal growth and their sporulation also influenced by various biochemical and physiological factors like, nutrients, ionic strength, pH, temperature, acid and alkaline conditions, light, aeration and water activity.

All living beings require food for the growth and reproduction for survival in nature. According to Hubballi et al., (2010) fungi secure food and energy from the inhibitory substrate upon which they live in the nature. Several researches have been done to evaluate the influence of different media components along with physiological parameters for the maximum growth and sporulation of fungi (Kim et al., 2005; Saha et al., 2008; Sharma and Sharma 2011). The media components are significantly important for fungal culture and study, along with important physiological parameters that lead the maximum sporulation (Kim et al., 2005). Microbial culture media can be of different type, accordingly to the nutritional growth requirements of the microorganisms. Microorganisms need about 10 macro elements namely (C, O, P, K, H, N, S, Mg Ca and Fe). In addition to macro elements, all microorganisms also require many microelements like (Mn, Zn, Co, Mo, Ni and Cu), which are mainly part of enzymes and cofactors. Microorganisms also require growth factors, which are organic compound (Basu et al., 2015).

The Nutritional requirement of fungi for growth and sporulation are generally not complex (Smith and Grula 1981). Some fungi need additional chemical, physical and nutritional parameters to achieve maximum growth and sporulation (Carlile et al., 2001). Kaul and Sumbali (1998) suggested that dermatophytic fungi grow well in the media rich in nitrogen and carbon contents.

The present study was conducted to evaluate the different culture broth for the growth and sporulation of filamentous fungi causing superficial infection in human beings.

MATERIAL AND METHODS

Selection of Suitable Liquid Medium

For the study of growth and sporulation of selected keratinophilic and dermatophytic fungi, following four media were selected.

- **Modified Sabouraud's Dextrose Medium (SDM)**

  Neopeptone - 9.0 gm

  Dextrose -20gm

  KH_2PO_4- 1.5gm

  $MgSO_4.7H_2O$-0.5gm

  Distilled water -1.0 liter

- **Richard's Medium (RM)**

  Sucrose- 50.0 gm

  KNO_3-10gm

  $MgSO_4.7H_2O$-2.50gm

  $FeCl_3$-0.02gm

  Distilled water-1.0 liter

- **Yeast Extract Medium (YEM)**

Yeast extract-4.0gm

Malt extract- 10.0gm

Dextrose- 4.0gm

Distilled water-1.0liter

- **Czapek's Medium (CzM)**

$NaNO_3$-2.0gm

$KHPO_4$-1.0gm

$MgSO_4$-0.5gm

KCl-0.5gm

$FeSO_4$-0.01gm

Sucrose-20.0gm

Distilled water-1liter

Fungal Culture

For the present investigation, four fungi namely *Trichophyton rubrum, T. terrestre, T. simii* and *Chrysosporium indicum* were selected. *T. rubrum and T. simii* were isolated from patients suffering from Tinea infection, SMS Medical college, Jaipur, while *T. terrestre* and *C. indicum* were isolated from soil sample through TO.KA.VA hair bating technique of Vanbreuseghem (1952).

Technique Used

The medium was prepared separately with a control 7.5 initial pH. In process 100 ml of liquid media was dispensed in 250 ml conical flask and sterilized in the autoclave at l21°C at 15 lbs pressure for 15 min. Pure culture of selected fungi was grown separately in the four different media for 15 days and on the 16th day, the mausoleum was harvested from each of flask by the usual method. Three replicates were used for each treatment. The pH of the culture filtrates was also recorded for each medium. Dry mat weight and amount of speculation were the criteria used for ascertaining the efficacy of a particular medium tested. The degree of speculation was determined before harvesting the mycelial mats, using standard methods as recommended by Wilson and Knight (1952) and Tuite (1962) as per the formula given below: **Number of spore/ml= Number of spores counted × Microscopic Field×1000**

Four Categories Were Made Accordingly

+1= poor

++= Fair

+++= Good

++++= Excellent

All the mycelial mats belonging to the three replicates of each treatment were pooled together in the same filter

paper and finally dried in the oven at 60ºC for two consecutive days. The Final weight was calculated.

Data Analysis

Results are given as mean ± standard error (S.E.) of N observations taken in three replicates (n = 3). Data sets were examined by one-way analysis of variance (ANOVA). P - values of less than 0.05 was considered significant.

RESULTS

A good medium should contain that compound in which, fungi can grow and sprout well and fast. In the present investigation, four different liquid culture media, namely Modified Sabouraud's Dextrose Medium (SDM), Yeast Extract Medium (YEM), Richard's Medium (RM) and Czapek's Medium (CzM) were tested for the better growth and sporulation of selected fungi. Data incorporated into table 1 represent the results of growth and sporulation of *T. rubrum* in different culture media. *T. rubrum* showed maximum sporulation on SDM and RM followed by YEM. The Maximum mycelial growth was achieved with SDM (0.275±0.020gm) and RM (0.273±0.001gm). CzM showed very poor growth and sporulation. The final pH of media changed variably in different media in contrast with an initial pH of media (7.5). It was 8 in SD, 8.8 inYEM, 6.5 in RM and 7.0 in CzM. *T. simii* showed best growth and sporulation in SDM followed by RM and YEM for mycelial growth. However, both showed the same rate of sporulations. CzM showed very poor growth and sporulaions. The initial pH maintained at 7.5 was changed after 15 day of growth. In case of SDM it remained unchanged while in YEM it increased (8). In RM (6.5) and CzM (7.3) the pH of media decreased than of initial pH (table 2). In case of *T. terrestre* (Table 3) maximum growth was reported in RM while maximum sporulation was seen when SDM and RM were applied (Table 3). YEM also showed good growth and sporulation. Final pH of the media shifted towards acidic side in the case of CzM (7.2) and RM (6.8) whereas it changed to alkaline side that is 8 in SD and 8.5 in YEM.

Maximum sporulation of *C. indicum* was achieved in RM and SDM, while excellent growth was reported in RM followed by YEM. SDM was placed third in fungal growth (Table 4). CzM also showed good growth and sporulation and placed fourth. In case of *C. indicum*, the final pH decreased in all the media tested.

DISCUSSIONS

The nature of particular medium has a great role to play in the growth and sporulation of fungi. Certain fungi have specific requirements but generally all fungi grow well in medium which are rich in nitrogen and carbon contents. The difference in the utilization of different carbon compounds is attributed to the presence or absence of specific adaptive, constitutive or hydrolyzing enzymes in fungal body. Different media have been suggested by various workers from time to time depending upon the nature of the fungi or the purpose of the study (Riker and Riker 1936; Sabouraud 1910).

In the present investigation, excellent speculation in all fungi was found in SDM among all media tested. Hence, for uniformity, SDM was chosen as the basal medium for the present study. In the case of *T. simii*, the best sporulation the growth was achieved when SDM was used. The same result was also obtained in the case of *T. rubrum* in which SDM and RM showed excellent speculation and growth. Garg (1974) who worked on keratinophilc fungi found Sabouraud Glucose Agar to be the most suitable medium. Sharma (1983) found SDA to be the most suitable for the overall growth and sporulation of keratinophilic fungi studied. She worked with *T. simii* and found similar results.

But in the case of *C. indicum* the maximum growth was obtained in Richard's Medium followed by Yeast Extract Medium, while the best sporulation was obtained in Sabouraud's Medium and Richard's medium. *C. indicum* was the only fungus that showed good growth in Czapek's media also whereas the other fungi showed very poor growth and sporualtion in this medium. *T. terrestre* showed the best sporulation in SDM and RM while the best growth was obtained only in Richard's Medium. Czapek's Medium showed very poor growth. An individual medium shows great role to play in the growth and sporulation of fungi. Jacques et al., (2002) studied the effect of liquid culture media on morphology, growth, propagule production, and pathogenic activity of the Hyphomycetes, *Metarhizium flavoviride* and found that the liquid medium possess excellent response. Sharma and Sharma 2011 studied different culture media for the growth and sporulation against selected fungi and found SDM, PDM and RM for the most favorable media of the growth and sporulation of dermatophytes and keratinophilic fungi. Gupta et al., (2013) evaluated the growth of keratinophilic fungi on five different culture media. Among them SDB found the best growth medium for all selected fungi followed by the PDA. Ingle (2014) also reported SDB as highest fungal biomass (0.609 g) producing medium against *Nomuraea rileyi* followed by the BCY broth (0.590 g). Khadim et al., (2015) also studied effect of different culture media like SDA, PDA, CMA, and YEA in growth rate of eight isolates of *T. rubrum.* Maximum growth was seen SDB. Kumawat et al., (2016) studied the influence of the liquid culture media (synthetic and natural), pH and temperature on the growth of mycelium and sporulation of a keratinophilic fungus *Arthroderma multifidum* (KU560574) isolated from soils of poultry farmhouse. Sabouraud's dextrose broth was found to be the most suitable medium for mycelial growth (1.229±0.12 GM) of *A. multifidum* due to its good and balanced nutrient content. Maximum sporulation was observed in the malt extract broth medium.

CONCLUSIONS

It was also recorded during the studies on different liquid media that, the final pH of media, after the completion of the experiments with the selected fungi shifted towards alkalinity when the initial pH of the media was 7.5. The increases in the pH of the culture media containing organic sources (peptones, amino acids) were probably due to the production of ammonia by the oxidative de-animation process as reported by Nickerson (1947) and Lilly and Barnett (1951). In other media, the shift in the final pH has been reported towards the acidic side. This may be due to the presence of more carbohydrate content along with peptone or other nitrogen sources. Lilly and Barnett (1951) expressed the view that this increase of alkalinity in carbohydrates containing media may be due to the production of acids from carbohydrates in the culture medium. On the CzM, the shift in the final pH was very little because of the considerable ratio of carbohydrate and nitrogen in the medium. Kumar and Bhadauria (2017) also recorded that the pH of the medium changed at the end of the incubation period where it floated towards the neutrality or an alkaline range from control range.

The present study will help to maintain the fungus in the laboratory condition for the preparation of inoculums, for different studies concerning control of the pathogen causing dermatophytic infections in human and animals.

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APPENDICES

Table 1: Average Dry Weight, Sporulation and Final Ph of Trichophyton Rubrum

Media	Initial pH	Final pH	Average Dry Weight(gm)	Sporulation
SabouraudsMedium	7.5	8	0.275±0.020	++++
Richard's Medium	7.5	6.5	0.273±0.001	++++
Yeast Extract Medium	7.5	8.8	0.235±0.005	+++
Czapek's Medium	7.5	7	0.082±0.006	+

Table 2: Average Dry Weight, Sporulation and Final Ph of Trichophyton Simii

Media	Initial pH	Final pH	Average dry weight(gm)	Sporulation
Sabouraud's Medium	7.5	7.5	0.320±0.004	++++
Richard's Medium	7.5	6.5	0.309±0.006	+++
Yeast Extract Medium	7.5	8	0.249±0.006	+++
Czapek's Medium	7.5	7.3	0.081±0.002	+

Table 3: Average Dry Weight, Sporulation and Final Ph of Trichophyton Terrestre

Media	Initial pH	Final pH	Average dry weight(gm)	Sporulation
Sabouraud's Medium	7.5	8	0.263±0.009	++++
Richard's Medium	7.5	6.8	0.306±0.046	++++
Yeast Extract Medium	7.5	8.5	0.252±0.005	+++
Czapek's Medium	7.5	7.2	0.069±0.002	+

Table 4: Average Dry Weight, Sporulation and Final pH of Chrysosporium Indicum

Media	Initial pH	Final pH	Average Dry Weight(gm)	Sporulation
Sabouraud's Medium	7.5	7	0.329±0.006	++++
Richard's Medium	7.5	6	0.739±0.007	++++
Yeast Extract Medium	7.5	6.5	0.373±0.003	+++
Czapek's Medium	7.5	6.5	0.186±0.009	++